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NOTE ON VITAL STAINING OF *ACTIAS LUNA* SILK

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Vital staining results with *Pieris rapae* (Linnaeus) and *Colias* species have been reported (Kolyer, 1965 and 1966). In *P. rapae,* the dye neutral red imparted a red tint to all stages, and Sudan black B gave bluish larvae, greenish-black pupae, and blue internal color in adults. Silkworms fed neutral red spin "bright red" cocoons (Edwards, 1921).

In the present work, the dyes fed to *Actias luna* (Linnaeus) larvae (5th instar) were neutral red, Colour Index No. 50040, total dye content 88%, and Sudan black B, Colour Index No. 26150, both obtained from Allied Chemical Corp., New York City. These were ground and blended with P-12 Davenite mica (325 mesh; Hayden Mica Co., Wilmington, Mass.) at 3 parts dye per 97 parts mica, and the blend was rubbed on the underside of hickory leaves at about 18 mg. blend/in.^{2} surface. Incidentally, with stems in water the leaves kept well below 80°F at about 50% rel. humidity but wilted rapidly at 85-90°F.

Neutral red showed toxicity and caused pronounced inhibition of growth, as noted for the butterfly species. While no mortality occurred among control larvae, of 16 larvae fed the dye for 2-8 days (followed by feeding undyed leaves if larvae hadn't died) only 2 survived to produce cocoons; these were fed dye for 2 days, at which point one began spinning while the other began a day after being transferred to undyed leaves. Success seemed to depend on feeding dye only long enough to saturate the body with the color (2 days, or even as little as 12 hours with voracious feeding) and on choosing larvae almost ready to pupate.

Four larvae were fed Sudan black B for 7 days and then transferred to undyed leaves. Only one survived and spun a cocoon. In this case, desorption of dye from the body was indicated; feeding on undyed leaves caused gradual loss of the deeper green shade given by the dye. Similar reversibility of neutral red has been noted for the wild silkworm *Attacus orizaba* (Edwards, 1921) as well as in the cited work with butterflies. The cocoon was uncolored like that of a control.

The two strongly-pink or rose colored cocoons from the neutral red experiment were opened to disclose dead larvae. After discarding the latter and picking all leaf fragments from the silk, the cocoons weighed 70 and 79 mg. Each cocoon was assayed for neutral red by triturating a sample (37-39 mg.) with 6 ml. of concentrated (37-38%) aqueous HCl, filtering, and measuring optical density at 725 millimicrons with a Bausch and Lomb Spectronic 20 Colorimeter. A calibration curve was constructed using known concentrations of dye in the HCl solution. The result was 1.3 ± 0.1% neutral red (as "total dye") in the cocoons. Experiments with *Attacus orizaba* (Edwards, 1921) have shown that the neutral red in the cocoon indeed is transferred to the sericin through the insect's body rather than being picked up externally during spinning.

LITERATURE CITED

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